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A New Series of M3 Muscarinic Antagonists Based on the 4-Amino-piperidine Scaffold

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Abstract—A series of 4-amino-piperidine containing molecules have been synthesized and structure–affinity relationship toward the M3-muscarinic receptor has been investigated. Chemical modulations provided molecules with K_i for the human M3-R up to 1 nM with variable selectivity (3- to 40-fold) over the human M2-R. Compounds **2** ($pA_2 = 8.3$, 8.6) demonstrates in vitro on guinea pig bladder and ileal strips potent anticholinergic properties and tissue selectivity.

The M3 muscarinic receptor (M3-R) plays a key role in the contraction of smooth muscle in a variety of organs. Ability to block the effect of the parasympathetic system and acetylcholine on post-junctional M3-R explains the therapeutic efficacy of anticholinergic drugs used to treat diseases associated with altered smooth muscle contractility such as, urinary urge incontinence and irritable bowel syndrome.[1] Unfortunately, the usefulness of current anticholinergic medication is limited by side effects such as tachycardia, dry mouth, constipation and blurred vision.[2] Selectivity towards M3 over M2 muscarinic receptors minimizes the risk of cardiovascular effects due to known involvement of M2-R in the regulation of cardiac rhythm. Nevertheless, requirement of M3-subtype selective antagonism remains controversial. Firstly, non cardiac systemic side effects (e.g., dry mouth) are prominent and are all M3-R mediated. Secondly, M2-R predominate in number over M3-R in smooth muscle and are thought to assist contraction by abrogating muscle relaxation induced by activation of the sympathetic system.[1,3,4] Thus, there is a great interest in finding muscarinic antagonists displaying tissue selectivity for the targeted organ.

In this communication, we describe the in vitro pharmacological profile of new *N*,*N*-disubstituted 4-amino-1-benzylpiperidine derivatives as muscarinic antagonists. Screening of our in house library identified compound **1** which was selected for further chemical modulation. These compounds were tested for muscarinic affinity using recombinant human muscarinic m3 and m2 receptors, and were thereafter tested for functional muscarinic receptor subtype or tissue selectivity in guinea pig urinary bladder, ileal smooth muscle (M3) and atrial muscle (M2) preparations.

Chemistry

The synthetic route[5] to the 4-substituted amino-piperidine derivatives is outlined in Scheme 1. Briefly, *N*-benzylated piperidone and the corresponding amine are dissolved in CH_2Cl_2 in the presence of titanium chloride in catalytic amounts, to give the imine derivatives which are then reduced using sodium borohydride in MeOH to the corresponding secondary amines. Compounds **1**–**25** are obtained by acylation using the corresponding acyl chlorides in the presence of potassium carbonate.

Results and Discussion

The synthesis of **1** (CAS No. 95869-86-6) and some related 4-amino-piperidines analogues has been published previously,[6] mainly as synthetic intermediates for the preparation of various potential therapeutic agents such as antiarrhytmic benzopyran derivatives,[7] anti-asthma benzimidazolone neurokinin 1 antagonists[8] and

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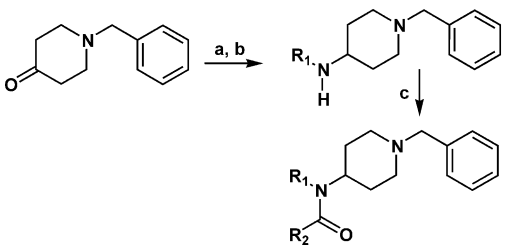

Scheme 1. Procedure for the synthesis of the series of 4-amino-piperidine derivatives: (a) R1-NH_2, CH_2Cl_2, $TiCl_4$, 0 °C; (b) $NaBH_4$, MeOH; (c) $CHCl_3$, K_2CO_3, R_2COCl.

therapeutic claims related to analgesia.[9] Thus, to the best of our knowledge, no publication has reported on the antimuscarinic properties of such compounds.

As seen in Table 1, the unsubstituted compound **1** (R1 = R2 = Ph) had an affinity of 25 nM (pK_i 7.6) for the M3-R. The introduction of a bromine (**2**) or a chlorine (**4**) atom at the *para* position of the phenyl ring in R1 increased the affinity for the M3-R almost 25-fold. The same trend was observed with a 2-thienyl moiety (**3** and **5** vs **16**) in R2. Whereas the phenyl ring in R2 could be replaced by a 2-thienyl moiety (**1** vs **16**), a slight 4-fold decrease in affinity for the M3-R was observed using a furyl moiety (**17** vs **1**; pK_i = 7.0 vs 7.6) as the replacing aromatic isosteric group.

Interestingly, the 4-Br/4-Cl-aryl substitutions in R2 (**18**, **19**, **20**) did not increase the affinity for the M3-R contrary to what was noted for the R1 position. Moreover, the substitution of the phenyl or thienyl ring at the R2 position with NO_2 (respectively, **21** vs **1**, **22** vs **3**), induces a decrease in affinity for the M3-R (pK_i = 6.1 vs 7.6 and 8.1 vs 8.7, respectively).

Introduction of a hydroxyl (**6**, pK_i = 8.6) or a OMe group (**7**, pK_i = 8.4) at the *para* position of the phenyl ring in R1 leads to compounds with slightly less affinity for the M3-R than the corresponding halogenated derivatives, but still more potent than the unsubstituted compound **1**. Compound **8**, bearing a 3,4-diCl aryl group in R1 displayed almost the same affinity as the corresponding 4-Cl derivative **4**. In contrast, **9**, bearing a 2,6-diCl aryl group in R1 is 150-fold less potent than compound **5**, the isosteric 2-thienyl derivative bearing a 4-Cl aryl group at the same position.

The replacement of the aryl moiety in R1 with a cyclobutyl group reduced at least 400-fold the affinity for the M3-R in both phenyl (**10** vs **1**) and 2-thienyl (**11** vs **16**) families. Moreover, a 30-fold reduction of affinity occurs when replacing the phenyl group in R1 by a 3-pyridyl moiety (**18**; pK_i = 7.5 vs **12**; pK_i = 6.0).

Interestingly, the replacement of the aryl moiety by a cyclo-butyl group in R2 (**13** vs **1**, **14** vs **2**) did not change the affinity for the M3-R. In contrast, replacing the cyclo-alkyl group in R2 by a methyl group (**15** vs **2**, pK_i = 6.8 vs 9.0) induces a considerable reduction (150-fold) in affinity.

Compounds bearing a $(CH2)_n$–Ph (n = 1,2) group in R1 (**25**) or R2 (**23**, **24**) were 3- to 10-fold less potent than **1**.

In summary, the following structure–affinity relationships toward the M3-R can be outlined:

- the R1 group in the 4-amino-piperidine scaffold is preferably an aromatic ring.
- Substituting the *para* position of the aryl group in the R1 position by halogen atoms (Br, Cl) or electron donating group (OMe) considerably increases the M3-R affinity whereas no change or even a decrease in affinity is observed when substituting the corresponding *meta* and *ortho* positions.
- Substituting the *para* position of the aryl group in R2 by halogens does not change the affinity for the M3-R contrary to what was observed in R1 position but the introduction of an electron-withdrawing group like NO_2 decreases the affinity for the M3-R.
- Only the R2 position allows the replacement of the aromatic ring by cyclo alkyl (e.g., cButyl) groups.
- Increasing the length of the spacer between the nitrogen atom of the amide link and the aryl groups in R1 and R2 significantly decreases affinity the M3-R.
- Replacement of the piperidinic *N*-benzyl group (results not shown) by H or Me groups induces a 400- to 1000-fold loss of affinity for the M3-R.

Table 1. The affinities for M2-R and M3-R were measured by the ability of the compounds to displace [^{3}H] *N*-methylscopolamine using membrane preparations from CHO-K1 cells expressing the human recombinant m2 and m3 muscarinic receptors[10]

	R1	R2	M3	M2	Bladder	Ileum	Atria
1	Ph	Ph	7.6	6.5			
2	4-Br-Ph	Ph	9.0	8.3	8.3	8.6	7.7
3	4-Br-Ph	2-Thienyl	8.7	7.6	7.5	8.0	6.8
4	4-Cl-Ph	Ph	8.9	8.1	7.9	8.1	7.9
5	4-Cl-Ph	2-Thienyl	8.4	7.3	7.4	7.5	7.0
6	4-OH-Ph	Ph	8.6	7.1	7.6	7.8	6.8
7	4-OMe-Ph	Ph	8.4	6.9	7.5	7.6	7.0
8	3,4-diCl-Ph	Ph	8.7	7.7	7.8	7.9	6.9
9	2,6-diCl-Ph	2-Thienyl	6.2	5.7	—	—	—
10	cButyl	Ph	++	++	—	—	—
11	cButyl	2-Thienyl	+	+	—	—	—
12	3-Py	4-Br-Ph	6.0	++	—	—	—
13	Ph	cButyl	7.8	6.6	—	—	—
14	4-Br-Ph	cButyl	8.8	7.7	7.9	7.9	7.5
15	4-Br-Ph	Me	6.8	6.4	—	—	—
16	Ph	2-Thienyl	7.6	6.7	—	—	—
17	Ph	2-Furyl	7.0	6.1	—	—	—
18	Ph	4-Br-Ph	7.5	6.2	—	—	—
19	Ph	4-Cl-Ph	7.6	6.2	—	—	—
20	4-Br-Ph	4-Cl-Ph	9.1	7.7	7.4	7.6	6.9
21	Ph	4-NO_2-Ph	6.1	5.4	—	—	—
22	4-Br-Ph	5-NO_2-2-thienyl	8.1	6.5	6.0	6.7	5.7
23	Ph	Ph-CH_2	7.6	6.5	—	—	—
24	Ph	Ph-CH_2-CH_2	7.1	6.1	—	—	—
25	Ph-CH_2	Ph	6.8	6.2	—	—	—

Results are expressed as pK_i or inhibition (+ <20%; ++ 20–50%) of the radioligand specific binding by 10 μM of the test compounds. Antagonism of M3-R and M2-R was measured by the ability of compounds to displace (pA_2) the concentration–response curve of carbachol on isolated guinea-pig urinary bladder, ileum[11] and paced left atria.[12]

Thus, the 4-amino-piperidine moiety can be considered as a good alternative to other reported M3-R muscarinic antagonist scaffolds.[13–17] Indeed, the main pharmacophoric elements allowing a good affinity for the M3-R can be defined as two aromatic groups (one replaceable by a cyclo-alkyl group) linked to a basic nitrogen via a piperidine containing spacer (Fig. 1). The basic nitrogen being generally linked to a lipophilic group (e.g., benzyl or isoprenyl). The SARs (structure–affinity relationships) related to these scaffolds are close to what has been published in this article for the 4-amino-piperidine scaffold.

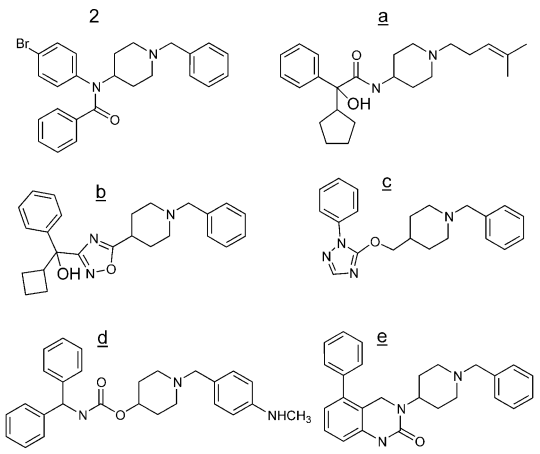

Figure 1. Structures of **2** and of other M3 antagonists with a 1,4-substituted piperidine in their backbone: a ,[13] b ,[14] c ,[15] d ,[16] e.[17]

It thus becomes clear that the functional as well as the tissue selective activity will definitely help to better define the therapeutic potential of such compounds.

As illustrated in Figure 2A, most of the present compounds have at least 3-fold greater affinity for the human M3-R over the M2-R. The most selective compounds are **22** (40 fold selectivity), **6** and **7** (32-fold selectivity). These compounds displayed a rather selective affinity for the muscarinic receptor, similar to that documented for the selective M3 antagonist Darifenacine.[18–20]

The functional antagonism of carbachol responses in guinea pig urinary bladder, ileal and atrial preparations

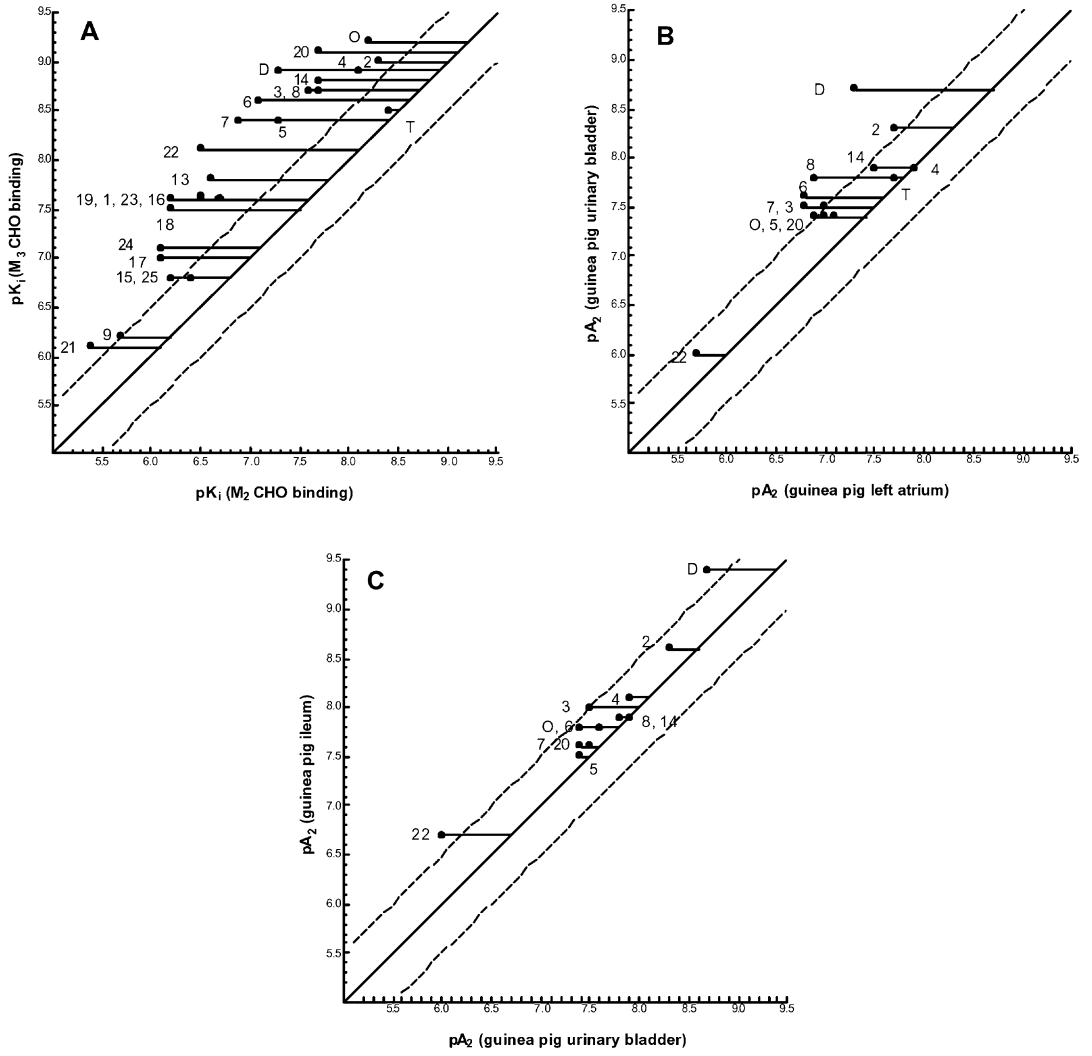

Figure 2. Analysis of data from Table 1. Values of Tolterodine (T), Darifenacine (D) and Oxybutynin (O) are from Newgreen et al.,[18] Gilberg et al.[19] and Wallis et al.,[20] respectively.

(Table 1 and Fig. 2) was evaluated for compounds displaying a pK_i value greater than 8.0 for the M3-R. Increasing concentrations of these compounds induced a parallel shift to the right of the concentration–response curve to carbachol on all three organs, indicating the competitive nature of the antagonism on both M3 and M2 muscarinic receptors.

Compound **2** was the most potent anticontractile agent on both the ileum ($pA_2 = 8.6$) and the bladder ($pA_2 = 8.3$). Anticontractile potency of compound **2** on the bladder lies between the reported pA_2 values[18,20] of Darifenacine ($pA_2 = 8.7$), Tolterodine (pA_2 bladder = 7.8) and Oxybutynin (pA_2 bladder = 7.4), which are drugs under development or already used for the treatment of irritable bowel syndrome and urinary incontinence.

Compounds **2**, **8**, **6**, **7** were 3- to 5-fold more potent on the bladder than on the atria (Fig. 2B). They behave in vitro as slightly more selective (bladder vs atria) than Tolterodine (pA_2 atria = 7.7, non-selective) and Oxybutynin (pA_2 atria = 7.1; 2-fold selectivity) but far less selective than Darifenacine (pA_2 Atria = 7.3; 25-fold selectivity). Compounds **22, 6** and **7** were thus functionally far less selective (2-, 6-, 3-fold, respectively) than expected from their binding profile. This discrepancy might be due to the experimental conditions (human versus guinea pig preparations, membrane versus tissue extract), which could affect molecular diffusion properties, thus changing compound distribution at the membrane M3-R vs the tissue M3-R. Most compounds were equiactive on the bladder and the ileum (selectivity ratio < 3; Fig. 2C) with the exception of compound **22** which was 5-fold more potent on the ileum than on the urinary bladder.

The most potent compound **2** failed (results not shown) to compete with radioactive ligands specific to the adenosine A_1, A_2, adrenergic α_1, α_2, and β, serotoninergic 5-HT_{1-7}, dopaminergic D_1, D_2 and histaminergic H_1, H_2, H_3 receptors at 10 μM indicating that it binds in a relatively selective manner to muscarinic receptors. Furthermore, on isolated rat aorta precontracted with 100 mM KCl,[21] compound **2** inhibited KCl induced contractions ($pD'2 = 4.2$ and 4.1, respectively) at a concentration range far exceeding that required for muscarinic mediated anticontractile effects in the bladder or ileum. This indicates that **2** is unlikely to possess calcium antagonist potential at relevant pharmacological doses.

Conclusion

Pharmacomodulation around the 4-amino-piperidine scaffold gave rise to potent M3-muscarinic receptor antagonists. Overall, this chemical family shows a binding selectivity for human M3 over M2 muscarinic receptors and functionally an increased anticontractile potential on the guinea pig bladder or ileum toward that displayed on the atria. Thus compound **2** is as potent anticontractile agent in vitro as clinical reference compounds. These results indicate that lead compounds derived from the 4-amido- or 4-sulfonamido-*N*-benzyl piperidine scaffold may have therapeutic potential for the treatment of urinary incontinence and/or irritable bowel syndrome.

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References and Notes

1. Eglen, R. M.; Choppin, A.; Watson, N. *Trends Pharmacol. Sci*. **2001**, *22*, 409.
2. Feinberg, M. *Drugs Aging* **1993**, *3*, 335.
3. Ehlert, F. J.; Sawyer, G. W.; Esqueda, E. E. *Life Sci*. **1999**, *64*, 387.
4. Hegde, S. S.; Eglen, R. M. *Life Sci*. **1999**, *64*, 419.
5. General synthesis procedure for **1**–**26**. *Intermediate amines*: the primary amine (3 equiv) and *N*-benzylpiperidin-4-one (1 equiv) are stirred in 100 mL of dry CH_2Cl_2. The mixture is cooled to 5 °C, and then a solution of 0.6 equiv of $TiCl_4$ in 20 mL of dry CH_2Cl_2 is added dropwise. The reaction is followed by TLC. When the ketone derivative is completely consumed MeOH is added followed by 4 equiv of $NaBH_4$. Stirring is maintained between 0 and 5 °C for at least 2 h. Solvent is evaporated in vacuo, and the residue triturated in water (100 mL). The resulting precipitate is filtered, and the filtrate extracted with AcOEt. The organic layer is extracted by a 10% aqueous solution of AcOH. The aqueous layer is alkalinized with K_2CO_3 up to pH 7–8. The product is then extracted with chloroform, the organic layer being dried with $CaCl_2$. The hydrochloride derivative solubilized in dry acetone is formed by adding Hcl dissolved in diethyl ether. The precipitate is filtered, dried, and recrystallized from acetone.

4-amino-piperidine derivatives: the secondary amine-hydrochloride (1 equiv) and K_2CO_3 (4 equiv) are heated under reflux in dry chloroform for 30 min. Then 3 equiv of acyl chloride diluted in chloroform are added dropwise. After 20 h of stirring at room temperature, the solvent is evaporated in vacuo. The residue dissolved in 50 mL of water and extracted with $CHCl_3$. The organic layer is washed with a 10% aqueous solution of AcOH to eliminate traces of secondary amine. The $CHCl_3$ layer is washed with an aqueous solution of K_2CO_3 and water, dried with $CaCl_2$ and evaporated in vacuo. The hydrochloride derivative is formed by adding HCl in diethyl ether to the amine solution in dried acetone. The precipitate is filtered, dried, and recrystallized from a mixture of acetone/diethyl ether (1:3). *N-(1-benzylpiperidin-4-yl)-N-(4-bromophenyl)-benzamide* (**2**): $C_{25}H_{25}BrN_2O$, HCl, M = 485.97 g mol^{-1}. Mp 241.5 °C. IR (KBr), ν (cm^{-1}): 1639 (CO); ^1H NMR (DMSO-d_6), δ (ppm): 1.7–2.1 (m, 4H, $C(CH_2)_2$), 3.1–3.4 (m, 4H, $N(CH_2)_2$), 4.2 (s, 2H, $CH_2\phi$), 4.8 (m, 1H, CH), 7.0–7.6 (m, 14H, aromatics), 10.8 (s, 1H, NH^+). ^{13}C NMR (DMSO-d_6), δ (ppm): 26.4 ($C(CH_2)_2$), 50.1–50.3 ($N(CH_2)_2 + CH$), 58.4 ($CH_2\phi$), 120.4–137.7 (C aromatics), 120.4, 129.4, 136.1, 137.7 (Cq), 169.2 (C=O). MS (ESI) $MH^+ = 449$ (100%); 451 (99%).
6. Janssen C. Patent application no. 1.344.366, 1962.
7. Barreau, M.; Hardy, J. C.; Martin, J. P.; Renault, C.; EP379,441, 1990.
8. Rémond, G.; Portevin, B.; Bonnet, J.; Canet, E.; Regoli, D.; De Nanteuil, G. *Eur. J. Med. Chem*. **1997**, *32*, 843.
9. Srulevitch, D. B.; Lien, E. J. *QSAR Drug Des*. **1989**, 377.
10. Buckley, N. J.; Bonner, T. I.; Buckley, C. M.; Brann, M. R. *Mol. Pharmacol*. **1989**, *35*, 469.

11. Posangi, J.; Zar, M. A.; Harris, J. B. *Br. J. Pharmacol.* **1992**, *106*, 307.
12. Lambrecht, G.; Feifel, R.; Wagner-Röder, M.; Strohmann, C.; Zilch, H.; Tacke, R.; Waelbroeck, M.; Christophe, J.; Boddeke, H.; Mutschler, E. *Eur. J. Pharmacol.* **1989**, *168*, 71.
13. Mitsuya, M.; Mase, T.; Tsuchiya, Y.; Kawakami, K.; Hattori, H.; Kobayashi, K.; Ogino, Y.; Fujikawa, T.; Satoh, A.; Kimura, T.; Noguchi, K.; Ohtake, N.; Tomimoto, K. *Bioorg. Med. Chem.* **1999**, *7*, 2555.
14. Mackensie, A. R.; Wood, A.; Bass, R. J. WO 9730994/970828, 1997
15. Courtemanche, G. V. C.; Bertin, J.; Defosse, G.; Bovy, P. R. FR 9901143, 1999.
16. Naito, R.; Takeuchi, M.; Morihira, K.; Hayakawa, M.; Ikeda, K.; Shibanuma, T.; Isomura, Y. *Chem. Pharm. Bull.* **1998**, *46*, 1286.
17. Muraoka, M.; Matsui, K.; Morishita, K.; Ohashi, N. EP 1 122 253 A1, 1999.
18. Newgreen, D. T.; Naylor, A. M. *Br. J. Pharmacol.* **1996**, *117*, 107, P.
19. Gilberg, P. G.; Sundquist, S.; Nivelbrant, L. *Eur. J. Pharmacol.* **1998**, *349*, 285.
20. Wallis, R. M.; Napier, C. M. *Life Sci.* **1999**, *64*, 395.
21. Polster, P.; Christophe, B.; Van Damme, M.; Houlliche, A.; Chatelain, P. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 593.